

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

The following claims will replace all prior versions and listings of claims in this application.

Claims 1–16 (Cancelled)

Claim 17 (Currently amended): A method for modifying glycosylation structures on glycoproteins expressed in a eukaryotic host cell comprising:

expressing in said host cell a recombinant nucleic acid encoding a polypeptide having an endomannosidase activity that is targeted to a vesicular compartment within the host cell, ~~wherein said nucleic acid encoding a polypeptide having an endomannosidase activity is selected from the group consisting of:~~

~~— (a) — a nucleic acid that hybridizes under stringent conditions to SEQ ID NO:1 or SEQ ID NO:3; and~~

~~— (b) — a nucleic acid that encodes a polypeptide that is at least 75% identical to SEQ ID NO:2 or SEQ ID NO:4.~~

Claim 18 (Previously presented): The method of claim 17 wherein the endomannosidase activity further comprises the activity of truncating Glc₁₋₃Man₉₋₅GlcNAc₂ to Man₈₋₄GlcNAc₂, wherein Glc α _{1,3}Man, Glc α _{2,3}Man or Glc α _{3,1,3}Man are removed.

Claim 19 (Previously presented): The method of claim 17 wherein the endomannosidase activity removes from a glucosylated glycan on proteins expressed in said host cell at least one glucose residue and at least one mannose residue.

Claim 20 (Previously presented): The method of claim 17 wherein the endomannosidase is targeted to the endoplasmic reticulum, the early, medial, late Golgi or trans Golgi network within the host cell.

Claim 21 (Original): The method of claim 17 wherein the endomannosidase is of host origin but has been modified by mutation, promoter strength or copy number to enhance activity.

Claim 22 (Previously presented): The method of claim 35 wherein the endomannosidase is secreted.

Claim 23 (Previously presented): The method of claim 17 wherein the host is a mammalian, plant, insect, fungal, yeast, algal or bacterial cell.

Claim 24 (Previously presented): The method of claim 17 wherein the host cell is from a eukaryote selected from the group consisting of *Pichia sp.*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium sp.*, and *Neurospora crassa*.

Claim 25. (Previously presented): The method of claim 17 wherein expression of the endomannosidase activity modifies a glucosylated glycoprotein that has bypassed the endoplasmic reticulum.

Claim 26 (Currently amended): The method of claim 17, wherein said nucleic acid is selected from the group consisting of:

- (a) SEQ ID NO: 1 or 3; ~~and~~
- (b) a nucleic acid sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2 or 4;
- (c) a nucleic acid that hybridizes under stringent conditions to SEQ ID NO:1 or SEQ ID NO:3; and
- (d) a nucleic acid that encodes a polypeptide that is at least 75% identical to SEQ ID NO:2 or SEQ ID NO:4.

Claim 27 (Previously presented): The method of claim 26 wherein the endomannosidase activity has optimal activity at a pH between about 5.2 and about 7.2.

Claim 28 (Previously presented): The method of claim 26 wherein the endomannosidase activity has optimal activity at a pH of about pH 6.2.

Claim 29 (Previously presented): The method of claim 26 wherein the encoded polypeptide hydrolyzes at least one glucose residue and at least one mannose residue on a Glc₁-₃Man₅GlcNAc₂, Glc₁-

3Man6GlcNAc₂, Glc₁-3Man7GlcNAc₂, Glc₁-3Man8GlcNAc₂, Glc₁-3Man9GlcNAc₂ or glucosylated higher mannan glycan.

Claim 30 (Previously presented): A eukaryotic host cell that produces modified glycosylation structures on proteins according to the method of claim 17.

Claim 31 (Previously presented): The method of claim 24 wherein the *Pichia* sp. is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, and *Pichia methanolica*.

Claim 32 (Previously presented): The method of claim 24 wherein the *Fusarium* sp. is selected from the group consisting of *Fusarium gramineum* and *Fusarium venenatum*.

Claim 33 (Previously presented): The method of claim 24 wherein the *Saccharomyces* sp. is *Saccharomyces cerevisiae*.

Claim 34 (Previously presented): The method of claim 24 wherein the *Kluyveromyces* sp. is *Kluyveromyces lactis*.

Claim 35 (Currently amended): A method for modifying glycosylation structures on glycoproteins expressed in a lower eukaryotic host cell comprising

expressing in the host cell a recombinant nucleic acid encoding a polypeptide having an endomannosidase activity, ~~wherein said nucleic acid encoding a polypeptide having an endomannosidase activity is selected from the group consisting of:~~

~~—— (a) —— a nucleic acid that hybridizes under stringent conditions to SEQ ID NO:1 or SEQ ID NO:3; and~~

~~—— (b) —— a nucleic acid that encodes a polypeptide that is at least 75% identical to SEQ ID NO:2 or SEQ ID NO:4.~~

Claim 36 (Previously presented): The method of claim 35 wherein the endomannosidase activity further comprises the activity of truncating Glc₁-3Man9-5GlcNAc₂ to Man8-4GlcNAc₂, wherein Glcα1,3Man, Glc2α1,3Man or Glc3α1,3Man are removed.

Claim 37 (Previously presented): The method of claim 35 wherein the endomannosidase activity removes from a glucosylated glycan on proteins expressed in said host cell at least one glucose residue and at least one mannose residue.

Claim 38 (Previously presented): The method of claim 35 wherein the endomannosidase is targeted to the endoplasmic reticulum, the early, medial, late Golgi, trans Golgi network or any vesicular compartment within the host cell.

Claim 39 (Cancelled)

Claim 40 (Previously presented): The method of claim 35 wherein the host is a fungal, yeast or algal cell.

Claim 41 (Previously presented): The method of claim 35 wherein the lower eukaryotic host cell is from a eukaryote selected from the group consisting of *Pichia sp.*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium sp.*, and *Neurospora crassa*.

Claim 42 (Previously presented): The method of claim 41 wherein the *Pichia sp.* is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, and *Pichia methanolica*.

Claim 43 (Previously presented): The method of claim 41 wherein the *Fusarium sp.* is selected from the group consisting of *Fusarium gramineum* and *Fusarium venenatum*.

Claim 44 (Previously presented): The method of claim 41 wherein the *Saccharomyces sp.* is *Saccharomyces cerevisiae*.

Claim 45 (Previously presented): The method of claim 41 wherein the *Kluyveromyces sp.* is *Kluyveromyces lactis*.

Claim 46 (Previously presented): The method of claim 41 wherein expression of the endomannosidase activity modifies a glucosylated glycoprotein that has bypassed the endoplasmic reticulum.

Claim 47 (Currently amended): The method of claim 35 wherein said nucleic acid is selected from the group consisting of:

- (a) SEQ ID NO: 1 or 3; ~~and~~
- (b) a nucleic acid sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2 or 4;
- (c) a nucleic acid that hybridizes under stringent conditions to SEQ ID NO:1 or SEQ ID NO:3; and
- (d) a nucleic acid that encodes a polypeptide that is at least 75% identical to SEQ ID NO:2 or SEQ ID NO:4.

Claim 48 (Previously presented): The method of claim 47 wherein the endomannosidase activity has optimal activity at a pH between about 5.2 and about 7.2.

Claim 49 (Previously presented): The method of claim 47, wherein the endomannosidase activity has optimal activity at a pH of about pH 6.2.

Claim 50 (Previously presented): The method of claims 47, wherein the encoded polypeptide hydrolyzes at least one glucose residue and at least one mannose residue on a Glc₁₋₃Man₅GlcNAc₂, Glc₁₋₃Man₆GlcNAc₂, Glc₁₋₃Man₇GlcNAc₂, Glc₁₋₃Man₈GlcNAc₂, Glc₁₋₃Man₉GlcNAc₂ or glucosylated higher mannan glycan.

Claim 51 (Previously presented): A lower eukaryotic host cell that produces modified glycosylation structures on proteins according to the method of claim 35.

Claim 52 (Previously presented): The method of claim 17 wherein said nucleic acid encoding a polypeptide having endomannosidase activity is ligated in-frame to a cellular targeting signal peptide.

Claim 53 (Previously presented): The method of claim 35 wherein said nucleic acid encoding a polypeptide having endomannosidase activity is ligated in-frame to a cellular targeting signal peptide.

Claim 54 (New): A method for modifying glycosylation structures on glycoproteins expressed in a eukaryotic host cell comprising:
expressing in said host cell a recombinant nucleic acid encoding a polypeptide having an endomannosidase activity that is targeted to a vesicular compartment within the host cell, wherein said

Application No.: 10/695,243
Amendment Date: January 23, 2007
Reply to Office Action of: August 9, 2006

nucleic acid encoding the polypeptide having an endomannosidase activity is selected from the group consisting of (a) SEQ ID NO: 1 or 3; and (b) a nucleic acid sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2 or 4.

Claim 55 (New): A method for modifying glycosylation structures on glycoproteins expressed in a lower eukaryotic host cell comprising

expressing in the host cell a recombinant nucleic acid encoding a polypeptide having an endomannosidase activity, wherein said nucleic acid encoding a polypeptide having an endomannosidase activity is selected from the group consisting of (a) SEQ ID NO: 1 or 3; and (b) a nucleic acid sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2 or 4.